The Effect of Freezing on the Biomarker Concentration in Platelet-Rich Plasma Releasate: A Pilot Study

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INTRODUCTION: Autologous platelet-rich plasma releasate (PRPr) is formed by activating platelets in platelet-rich plasma to yield a high concentration of cytokines and chemokines that are used to enhance healing of a variety of musculoskeletal tissues. Activated platelets are capable of releasing a wide variety of cytokines and chemokines that can initiate healing, but the mechanism of action on the repair process is unknown and requires further investigation (1). Even though PRPr has shown to mediate inflammation and induces cell migration in vitro (1, 2) as well as reduce pain and improve patient function (3), clinicians and researchers are observing PRPr’s effects under fresh and frozen conditions. Roffi, et al tested the effects of freeze-thaw to fresh PRPr on IL-1βta, HGF, PDGF AB/BB, TGF-β1, and VEGF and found significantly lower TGF-β1 and PDGF-AB/BB, while IL-1β and HGF significantly increased after the freeze-thaw process (4). There is little knowledge on the effect of the freeze-thaw process in terms of the wide variety cytokine and chemokine concentrations. The purpose of this study was to quantify and compare cytokine and chemokine concentrations between fresh whole blood (WB), platelet-poor plasma (PPP), inactivated platelet-rich plasma (PRP), PRPr and PRPr-F.

METHODS: Using techniques from an IRB-approved protocol, three 60 mL syringes were prefilled with 5 mL of anti-coagulant citrate dextrose solution (ACD-A). Three healthy donors voluntarily donated 55 mL of peripheral venous WB via intra-venous catheter, and WB samples were immediately processed using the Angel System (Arthrex, Naples, Florida, USA) PRP processor at 1% hematocrit. PRPr was prepared with 1.67 mL of isolated PRP and 90 μL of 10% calcium chloride (w/v) in a 10 mL vacutainer for 30 minutes. The clots were retracted from the vacutainer following supernatant releasate formation. Blood was separated into five fractions including, WB, PPP, PRP, PRPr, and PRPr-F. The PRPr-F was stored at -80°C for 5 days and was then thawed in a 37°C water bath. Commercial Luminex kits were used according to manufacturer’s instructions for protein content: Milliplex MAP Human Cytokine 38-plex kit, Milliplex MAP Human Cytokine 3-plex kit, Milliplex MAP Human MMP Panel 1 kit, Milliplex MAP Human MMP Panel 2 kit, and Milliplex MAP TGF-β1, 1, 2, 3 kit (Millipore, Billerica, Massachusetts, USA). The data from the five groups were expressed as the mean ± standard deviation of three replicates. Statistical differences were determined using t-test and P<0.05 was considered statistically significant.

RESULTS: WB had lower concentrations of IL-13 compared to all other blood fractions and had significantly lower concentrations of MMP-1a, TNF-α, and MCP-3 compared to PRPr-F (Figure 3). However, TGF-β1 and 2 were lowest in WB compared to PRP, PRPr and PRPr-F, while etoxatin and MCP-1 were significantly higher in WB compared to all other blood fractions (Figures 2 & 3). PPP had significantly lower concentrations of MMP-13 compared to PRP, PRPr and PRPr-F, while MMP-3 and MMP-12 were significantly higher in PRPr-F compared to PRP and PRPr (p<0.05) (Figure 1). In addition, etoxatin, MCP-3 and IL-13 were increased in PRPr-F compared to PRP (p=0.03, p<0.05, p=0.04), and IL-8 was significantly higher in PRPr-F compared to all other blood fractions. We only observed higher concentrations of IL-13 and lower concentrations of TGF-β2 in PRPr compared to PRP (p=0.08, p=0.05) (Figure 2 & 3). IL-2, 3, 6, and 7 and TGF-β3 were not detectable in any of the samples. There were no significant changes in other assayed growth factors, cytokines, or chemokines.

DISCUSSION: We observed significant concentrations of specific pro-inflammatory and degenerative tissue factors in every PRP fraction except PRPr. MMPs are known to cause matrix degeneration and induce inflammation in various musculoskeletal tissues (5, 6). Pro-inflammatory factors are also capable of activating MMPs to initiate tissue degeneration (7). Further studies with a larger sample size are warranted to elucidate the effects of freezing on cytokine and chemokine concentrations in PRPr.

SIGNIFICANCE: We observed elevated concentrations of pro-inflammatory and degenerative factors in PRP-F compared to PRPr, which may affect the clinical and translational applications of PRPr.

ACKNOWLEDGMENTS: Arthrex, Naples, FL USA

REFERENCES:

IMAGES:

ORS 2017 Annual Meeting Poster No.0362